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=> s (cellulose binding domain or cellulose binding module) and protein purification
L1          83 (CELLULOSE BINDING DOMAIN OR CELLULOSE BINDING MODULE) AND PROTE
               IN PURIFICATION

=> dup rem l1
PROCESSING COMPLETED FOR L1
L2          71 DUP REM L1 (12 DUPLICATES REMOVED)

=> s l2 and soluble protein
L3           2 L2 AND SOLUBLE PROTEIN

=> d l3 1-2 ibib ab

L3   ANSWER 1 OF 2  EMBASE  COPYRIGHT (c) 2007 Elsevier B.V. All rights
     reserved on STN
ACCESSION NUMBER:   2005073845  EMBASE
TITLE:              Effects of the PT region of EngD and HLD of CbpA on
                    solubility, catalytic activity and purification
                    characteristics of EngD-CBD(CbpA) fusions from Clostridium
                    cellulovorans.
AUTHOR:             Yeh M.; Craig S.; Lum M.-G.; Foong F.C.
CORPORATE SOURCE:   F.C. Foong, Sch. of Biotech. and Biomol. Sci., University
                    of New South Wales, Sydney, NSW 2052, Australia.
                    f.foong@unsw.edu.au
SOURCE:             Journal of Biotechnology, (30 Mar 2005) Vol. 116, No. 3,
                    pp. 233-244.
                    Refs: 45
                    ISSN: 0168-1656  CODEN: JBITD4
PUBLISHER IDENT.:   S 0168-1656(04)00550-4
COUNTRY:            Netherlands
DOCUMENT TYPE:      Journal; Article
FILE SEGMENT:       004     Microbiology: Bacteriology, Mycology, Parasitology
                            and Virology
LANGUAGE:           English
SUMMARY LANGUAGE:   English
ENTRY DATE:         Entered STN: 24 Feb 2005
                    Last Updated on STN: 1 Feb 2007
AB   Chimeric proteins combining the catalytic N-terminal region of native EngD
     with its proline-threonine-threonine (PT) linker region, hydrophilic
     domain (HLD) and cellulose binding domain
     (CBD) of cellulose binding protein A (CbpA) from Clostridium cellulovorans
```

were constructed, expressed, and analyzed. The chimeric proteins with CBD(CbpA) all demonstrated strong affinity to Avicel. The chimeric protein with the PT region of EngD and the HLD had the best catalytic activity and the highest estimated percentage of soluble protein amongst the chimeric proteins. Native EngD and two of the chimeric proteins (EngD-PT-HLD-CBD and EngD-CBD) were purified and their characteristics analyzed. Their binding affinities to Avicel as well as their enzymatic activities against various substrates were found to be consistent with the results we saw from protein lysate samples, which was good binding to Avicel but a decrease in solubility and catalytic activities in chimeric proteins without PT and/or HLD. The reasons for these are discussed. These fusion proteins may be important in applications, such as immobilization to solid cellulose substrate for purification of proteins and enrichment/aggregation of protein complexes.

L3 ANSWER 2 OF 2 EMBASE

ACCESSION NUMBER: 2000394266 EMBASE
TITLE: Alpha-amylase inhibitors selected from a combinatorial library of a cellulose binding domain scaffold.
AUTHOR: Lehtio J.; Teeri T.T.; Nygren P.-A.
CORPORATE SOURCE: P.-A. Nygren, Department of Biotechnology, Royal Institute of Technology, SE-100 44 Stockholm, Sweden. perake@biochem.kth.se
SOURCE: Proteins: Structure, Function and Genetics, (15 Nov 2000) Vol. 41, No. 3, pp. 316-322.
Refs: 48
ISSN: 0887-3585 CODEN: PSFGEY
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology: Bacteriology, Mycology, Parasitology and Virology
LANGUAGE: English
SUMMARY LANGUAGE: English
ENTRY DATE: Entered STN: 13 Dec 2000
Last Updated on STN: 13 Dec 2000

AB A disulfide bridge-constrained cellulose binding domain (CBD(WT)) derived from the cellobiohydrolase Cel7A from Trichoderma reesei has been investigated for use in scaffold engineering to obtain novel binding proteins. The gene encoding the wild-type 36 aa CBD(WT) domain was first inserted into a phagemid vector and shown to be functionally displayed on M13 filamentous phage as a protein III fusion protein with retained cellulose binding activity. A combinatorial library comprising 46 million variants of the CBD domain was constructed through randomization of 11 positions located at the domain surface and distributed over three separate .beta.-sheets of the domain. Using the enzyme porcine alpha-amylase (PPA) as target in biopannings, two CBD variants showing selective binding to the enzyme were characterized. Reduction and iodoacetamide blocking of cysteine residues in selected CBD variants resulted in a loss of binding activity, indicating a conformation dependent binding. Interestingly, further studies showed that the selected CBD variants were capable of competing with the binding of the amylase inhibitor acarbose to the enzyme. In addition, the enzyme activity could be partially inhibited by addition of soluble protein, suggesting that the selected CBD variants bind to the active site of the enzyme. (C) 2000 Wiley-Liss, Inc.

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=> s l2 and (xylanase or enterokinase)
L4          12 L2 AND (XYLANASE OR ENTEROKINASE)

=> d l4 1-12 ibib ab
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L4 ANSWER 1 OF 12 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
ACCESSION NUMBER: 2003:332788 BIOSIS
DOCUMENT NUMBER: PREV200300332788
TITLE: Purification and characterization of five cellulases and one xylanase from Penicillium brasilianum IBT 20888.
AUTHOR(S): Jorgensen, Henning; Eriksson, Torny; Borjesson, Johan; Tjerneld, Folke; Olsson, Lisbeth [Reprint Author]
CORPORATE SOURCE: Center for Process Biotechnology, BioCentrum-DTU, Technical University of Denmark, Building 223, DK-2800, Kongens Lyngby, Denmark
lo@biocentrum.dtu.dk
SOURCE: Enzyme and Microbial Technology, (12 June 2003) Vol. 32, No. 7, pp. 851-861. print.
CODEN: EMTED2. ISSN: 0141-0229.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 16 Jul 2003
Last Updated on STN: 16 Jul 2003

AB The filamentous fungus Penicillium brasilianum IBT 20888 was cultivated on a mixture of 30 g l-1 cellulose and 10 g l-1 xylan for 111 h and the resulting culture filtrate was used for protein purification. From the cultivation broth, five cellulases and one xylanase were purified. Hydrolysis studies revealed that two of the cellulases were acting as cellobiohydrolases by being active on only microcrystalline cellulose (Avicel). Three of the cellulases were active on both Avicel and carboxymethyl cellulose indicating endoglucanase activity. Two of these showed furthermore mannanase activity by being able to hydrolyze galactomannan (locust bean gum). Adsorption studies revealed that the smaller of the two enzymes was not able to bind to cellulose. Similarity in molecular mass, pI and hydrolytic properties suggested that these two enzymes were identical, but the smaller one was lacking the cellulose-binding domain or an essential part of it. The basic xylanase (pI>9) was only active towards xylan. Two of the purified cellulases with endoglucanase activity were partly sequenced and based on sequence homology with known enzymes they were classified as belonging to families 5 and 12 of the glycosyl hydrolases.

L4 ANSWER 2 OF 12 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
ACCESSION NUMBER: 1999:153763 BIOSIS
DOCUMENT NUMBER: PREV199900153763
TITLE: Purification of a fusion protein using the family VI cellulose-binding domain of Clostridium stercorarium XynA.
AUTHOR(S): Sakka, Kazuo [Reprint author]; Karita, Shuichi; Kimura, Tetsuya [Reprint author]; Ohmiya, Kunio [Reprint author]
CORPORATE SOURCE: Fac. Bioresources, Mie Univ., Tsu 514, Japan
SOURCE: Laskin, A. I. [Editor]; Li, G.-X. [Editor]; Yu, Y.-T. [Editor]. Ann. N. Y. Acad. Sci., (1998) pp. 485-488. Annals of the New York Academy of Sciences; Enzyme engineering XIV. print.
Publisher: New York Academy of Sciences, 2 East 63rd Street, New York, New York 10021, USA. Series: Annals of the New York Academy of Sciences.
Meeting Info.: Fourteenth International Enzyme Engineering Conference. Beijing, China. October 12-17, 1997. Engineering Foundation, New York.
CODEN: ANYAA9. ISSN: 0077-8923. ISBN: 1-57331-149-9 (cloth), 1-57331-150-2 (paper).
DOCUMENT TYPE: Book
Conference; (Meeting)
Book; (Book Chapter)
Conference; (Meeting Poster)
Conference; (Meeting Paper)

LANGUAGE: English
ENTRY DATE: Entered STN: 16 Apr 1999
Last Updated on STN: 14 May 1999

L4 ANSWER 3 OF 12 BIOTECHDS COPYRIGHT 2007 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2005-11360 BIOTECHDS
TITLE: Purifying heterologous protein of interest by eluting a cellulose binding module
(CBM)-protease from a polysaccharide matrix by adjusting conditions affecting the release of the CBM-protease off the matrix;
for use in recombinant protein preparation and pharmaceutical industry
AUTHOR: MANTYLA E; ORVAR B L
PATENT ASSIGNEE: ORF LIFTAEKNI EHF
PATENT INFO: WO 2005021764 10 Mar 2005
APPLICATION INFO: WO 2004-IS11 27 Aug 2004
PRIORITY INFO: US 2003-497935 27 Aug 2003; IS 2003-6929 27 Aug 2003
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2005-214578 [22]
AB DERWENT ABSTRACT:
NOVELTY - Purifying a heterologous protein of interest comprises contacting the solution of cellulose binding module (CBM)-protease, free CBM and heterologous protein of interest to a polysaccharide matrix, and eluting the CBM-protease from the matrix by adjusting conditions affecting the release of the CBM-protease off the matrix.

DETAILED DESCRIPTION - Purifying a heterologous protein of interest comprises: (a) providing a fusion protein comprising the heterologous protein fused to a CBM intercepted by a proteolytic cleavage site; (b) contacting the fusion protein with a functional protease fused to a CBM, at conditions facilitating proteolytic cleavage by the protease, to cleave the CBM from the heterologous protein of interest; (c) contacting the solution of CBM-protease, free CBM and heterologous protein of interest to a polysaccharide matrix, under conditions where the CBM-protease and free CBM binds to the polysaccharide matrix and where the heterologous protein of interest is not retained on the polysaccharide matrix; (d) separating the non-bound heterologous protein of interest from the polysaccharide matrix; (e) washing the polysaccharide matrix with the bound CBM-protease and CBM, with one or more suitable aqueous solutions; (f) eluting the CBM-protease from the matrix by adjusting conditions effecting the release of the CBM-protease off the matrix; and (g) optionally reconditioning the eluted CBM-protease, to retain its affinity to the polysaccharide matrix, so that the reconditioned CBM-protease can be re-used for subsequent repetition of the process of (a)-(g).

BIOTECHNOLOGY - Preferred Method: In the method above, the protease fused to CBM is from an enterokinase, tobacco etch virus (TEV) protease, factor X, or thrombin. The protease is mammalian enterokinase (EK) or an enterokinase active part. The EK comprises a bovine EK catalytic domain (EKc). It is encoded by the nucleic acid comprising a fully defined 705 bp sequence (SEQ ID NO. 2) given in the specification. The protease fused to CBM and the heterologous protein fused to a CBM intercepted by a proteolytic cleavage site are obtained separately by a method for production and purification of a soluble heterologous fusion protein comprising a cellulose binding module, from transgenic plants or transgenic plant cells expressing the fusion protein, comprising: (a) disrupting the transgenic plant material; (b) adding an extraction liquid to the plant material, thus creating a mixture of soluble and insoluble plant material, to extract the soluble fusion protein from the disrupted plant material to the liquid phase to obtain a protein extract; (c) separating the insoluble plant material, comprising cell-wall material and solids, from the protein extract comprising the fusion protein of interest; (d)

contacting the protein extract to a polysaccharide matrix which binds to the fusion protein; (e) washing the matrix with the bound fusion protein with one or more suitable aqueous solutions; and (f) eluting the fusion protein from the polysaccharide matrix by adjusting conditions effecting the release of the fusion protein from the matrix, thus obtaining the soluble heterologous fusion protein substantially purified. The separation comprises a method selected from expanded bed adsorption (EBA), packed mode chromatography, precipitation, filtration, centrifugation, or its combination. The affinity binding to the polysaccharide matrix comprises a chromatography step. Steps (c) and (d) are performed simultaneously in a combined single step. Combining (c) and (d) in a process step comprises expanded bad adsorption with a polysaccharide matrix, as a measure for simultaneous separation of cell-wall material and solids from the protein extract and affinity binding of the CBM-fusion protein onto the polysaccharide matrix. The polysaccharide matrix comprises cellulose, where the cellulose is pharmaceutical cellulose. Preferably, the cellulose is Avicel (RTM). Reconditioning the eluted CBM-protease involves neutralization, and/or removal from the CBM protease eluent of agents that affect the release of CBM from the polysaccharide matrix. It also comprises neutralization or removal from the eluent of carbohydrates such as saccharides. The fusion protein comprising the heterologous protein of interest is expressed and retrieved from a transgenic plant or plant cell or by transient expression in a plant, plant tissue, or a plant cell. The transgenic plant or plant cell is selected from dicotyledonous plants or monocotyledonous plants. Preferably, the plant cell or transgenic plant is selected from tobacco, rapeseed, soybean, alfalfa, lettuce, barley, maize, wheat, oat, or rice. The CBM fused to the heterologous protein and the CBM fused to the protease are heat-stable and remain soluble at elevated temperatures. One or both of the CBMs are a CBM encoded by a region of the xylanase10A gene from Thermotoga maritima. One or both of the CBMs are encoded by a sequence comprising a fully defined 564 bp sequence (SEQ ID NO. 1) given in the specification, or a sequence encoding the same amino acid sequence or an amino acid sequence with substantial sequence identity to the sequence.

USE - The method is useful for purifying a heterologous protein of interest. It is useful for high-scale production of purified recombinant proteins from plants, plant-derived tissue, or plant cells.

ADVANTAGE - The method provides a novel process of separating CMB-fusion proteins from biomass such as plant-derived cellulosic material, with fewer processing steps involved, taking advantage of a safer and more economical affinity chromatography principle amenable for use with the pharmaceutical industry.(37 pages)

L4 ANSWER 4 OF 12 BIOTECHDS COPYRIGHT 2007 THE THOMSON CORP. on STN
ACCESSION NUMBER: 1999-14423 BIOTECHDS
TITLE: Purification of a protein;
by recombinant expression as a fusion protein with Clostridium stercorarium cellulose binding domain
PATENT ASSIGNEE: Toyobo
LOCATION: Japan.
PATENT INFO: JP 11225763 24 Aug 1999
APPLICATION INFO: JP 1998-29410 12 Feb 1998
PRIORITY INFO: JP 1998-29410 12 Feb 1998
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
OTHER SOURCE: WPI: 1999-521079 [44]
AB A means of purifying a protein is claimed. It involves the use of a solution containing a cellulose binding domain (CBD) derived from an enzyme capable of cellulose degradation, which is reacted with a cellulose adsorbent which adsorbs the target protein. This is then treated with a saccharide to elute the protein from the adsorbent. Also claimed is a fusion protein consisting of a protein to be purified linked to a CBD of a cellulose degrading

enzyme, and optionally also a given protein sequence and a cleavage site. The claims also cover a recombinant DNA encoding the fusion protein, a vector containing that DNA, a host cell transformed by the protein, and a means of producing the fusion protein by culturing that host cell. These are used to produce and purify a recombinant protein efficiently. The CBD is preferably derived from an endo-1,4-beta-D-xylanase (EC-3.2.1.8) produced by Clostridium stercorarium. (12pp)

L4 ANSWER 5 OF 12 BIOTECHDS COPYRIGHT 2007 THE THOMSON CORP. on STN
ACCESSION NUMBER: 1998-00342 BIOTECHDS
TITLE: Purification of the Ruminococcus albus endoglucanase-IV using a cellulose-binding domain as an affinity tag;
Clostridium stercorarium endo-1,4-beta-D-xylanase -A cellulose binding domain
AUTHOR: Karita S; Kimura T; Sakka K; Ohmiya K
CORPORATE SOURCE: Univ.Mie
LOCATION: Center for Molecular Biology and Genetics, Mie University, Tsu 514, Japan.
SOURCE: J.Ferment.Bioeng.; (1997) 84, 4, 354-57
CODEN: JFBIEX
ISSN: 0922-338X
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The gene encoding the single cellulose binding domain-II (CBD-II) of Clostridium stercorarium endo-1,4-beta-D-xylanase-A (EC-3.2.1.8) was fused to the egIV endoglucanase-IV (EC-3.2.1.4) gene from Ruminococcus albus, and the fusion protein expressed in Escherichia coli JM109. The CBD-II was amplified by polymerase chain reaction using DNA primers, the amplified fragment digested and ligated into plasmid pRAII to yield plasmid pCsCBD2, which encoded the fusion protein. E. coli harboring the plasmid were grown at 37 deg of Luria broth supplemented with 100 ug/ml ampicillin. A cell-free extract was prepared by sonication and protein concentrations and cellulase activities determined. The fusion protein was purified 15-fold using ball-milled cellulose (BMC) as an affinity matirix, with a 39% yield of the protein. The single step purification was possible as a result of the different affinities of CBD for BMC and cellobiose. The linker region between the two components of the protein could be cleaved by non-specific proteolysis, e.g. using trypsin. The CBD-II was a useful affinity tag for protein purification. (17 ref)

L4 ANSWER 6 OF 12 BIOTECHDS COPYRIGHT 2007 THE THOMSON CORP. on STN
ACCESSION NUMBER: 1997-02830 BIOTECHDS
TITLE: Comparison of the adsorption properties of a single-chain antibody fragment fused to a fungal or bacterial cellulose-binding domain;
fusion protein production for use in protein purification, enzyme purification or enzyme immobilization
AUTHOR: Reinikainen T; Takkinen K; Teeri T T
CORPORATE SOURCE: VTT
LOCATION: VTT Biotechnology and Food Research, P.O. Box 1500, FIN-02044 VTT, Finland.
SOURCE: Enzyme Microb.Technol.; (1997) 20, 2, 143-49
CODEN: EMTED2
ISSN: 0141-0229
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Trichoderma reesei cellobiohydrolase (CBHI, EC-3.2.1.91) and Cellulomonas fimi cellulase (EC-3.2.1.4)-endo-1,4-beta-D-xylanase (Cex, EC-3.2.1.1) both have distinct C-terminal cellulose-binding domains (CBDs) which belong to different CBD sequence families. Fusion proteins comprising a single chain antibody fragment (OxscFv) against 2-phenyloxazolone fused to either of the 2 CBDs were expressed in

Escherichia coli RV308 or WCM105. The binding activities of the fusion proteins were studied on different cellulosic substrates. The CBD-Cex bound the fusion protein to cellulose more effectively than the CBD-CHBI. Once immobilized, both fusion proteins could be eluted from the cellulose only with denaturing agents or very low or high pH. Both fusion proteins retained equally well their ability to bind the hapten recognized by their antibody part. A CBD can be fused genetically to a protein of interest for specific binding to a cheap and versatile cellulose matrix for use in purification or immobilization of a variety of recombinant proteins and enzymes. (44 ref)

L4 ANSWER 7 OF 12 BIOTECHDS COPYRIGHT 2007 THE THOMSON CORP. on STN
ACCESSION NUMBER: 1996-10215 BIOTECHDS
TITLE: Interactions of cellulases from Cellulomonas fimi with cellulose;
cellulase catalytic domain and cellulose-binding domain interaction (conference paper)
AUTHOR: Din N; Coutinho J B; Gilkes N R; Jervis E; Kilburn D G; Miller Jr R C; Ong E; Tomme P; Warren R A J
CORPORATE SOURCE: Univ.British-Columbia
LOCATION: Department of Microbiology and Immunology, and Protein Engineering Network of Centres of Excellence, University of British Columbia, Vancouver, B.C. Canada V6T 1Z3.
SOURCE: Prog.Biotechnol.; (1995) 10, 261-70
CODEN: PBITE3
ISSN: 0921-0423
Carbohydrate Bioengineering, International Conference, Elsinore, Denmark, 23-26 April, 1995.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The 4 cellulases (endoglucanases, EC-3.2.1.4), 2 cellobiohydrolases (EC-3.2.1.91), endo-1,4-beta-D-xylanase (EC-3.2.1.8) and a mixed function exoglucanase-xylanase of Cellulomonas fimi are modular proteins comprising 2-6 domains. All contain a catalytic domain (CD) and a cellulose-binding domain (CBD) that function independently when separated by proteolysis or genetic engineering. The CDs have weak affinity for substrate, relative to the CBDs, and catalyze hydrolysis of glycosidic bonds with inversion or retention of anomeric configuration. The family II CBDs adsorb to both crystalline and amorphous cellulose (except for xylanase-D CBD which adsorbs only to crystalline cellulose). The family IV CBD from endoglucanase CenC adsorbs only to amorphous cellulose. Adsorption is strongly dependent on aromatic amino acids, especially tryptophans, which are conserved in nearly all family II CBDs. The endoglucanase CenA CBD has a disruptive effect on cotton fibers. The binding of family II CBDs to cellulose is stable enough for them to be used as affinity tags for protein purification and enzyme immobilization. (54 ref)

L4 ANSWER 8 OF 12 BIOTECHDS COPYRIGHT 2007 THE THOMSON CORP. on STN
ACCESSION NUMBER: 1995-03038 BIOTECHDS
TITLE: Separations in biotechnology;
protein purification (conference report)
AUTHOR: Chaudhuri J B
CORPORATE SOURCE: Univ.Bath
LOCATION: School of Chemical Engineering, University of Bath, Claverton Down, Bath, BA2 7AY, UK.
SOURCE: Trends Biotechnol.; (1995) 13, 1, 12-14
CODEN: TRBIDM
ISSN: 0167-9430
Separations for Biotechnology, 3rd International Conference, Reading, UK, 12-15 September, 1994.
DOCUMENT TYPE: Journal

LANGUAGE: English

AB Recent developments in the field of separations for biotechnology were presented, including: downstream processing; bioseparations; single-stage processes; process-scale operations; advances in separation processes; and affinity separations. Examples discussed included: use of a cellulose-binding domain affinity tail for affinity chromatography; use of chaperone heat shock protein-10 and -60 for improved protein folding in vitro; use of aq. two-phase system phase partitioning; temp.-induced phase separation; anion-exchange chromatography on DEAE-Spherodex for acidic protease purification; rate-zonal centrifugation; alkyl phenyl ether by Mycobacterium sp. M156 in a reversed micelle system, used in purification; use of hydrocyclones for recovery and dewatering of microbial suspensions; automated displacement chromatography; amphotericin-B purification by ionexchange chromatography; spray-drying as a final step in Trichoderma reesei endo-1,4-beta-D-xylanase (EC-3.2.1.8) purification; scale-up and process optimization; molecular imprinting for adsorbent construction; and affinity chromatography. (0 ref)

L4 ANSWER 9 OF 12 BIOTECHDS COPYRIGHT 2007 THE THOMSON CORP. on STN
ACCESSION NUMBER: 1995-00734 BIOTECHDS
TITLE: Production and purification of recombinant protein; Trichoderma reesei cellulase or cellobiohydrolase cellulose-binding domain affinity tail fusion protein cleavage using protease
PATENT ASSIGNEE: Toyo-Eng.
PATENT INFO: JP 06277088 4 Oct 1994
APPLICATION INFO: JP 1993-71559 30 Mar 1993
PRIORITY INFO: JP 1993-71559 30 Mar 1993
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
OTHER SOURCE: WPI: 1994-353764 [44]

AB A recombinant protein may be produced as a fusion protein with an affinity tail, followed by purification from a cell extract by affinity chromatography. The affinity tail is preferably a cellulase (EC-3.2.1.4) EGI or EGIII or cellobiohydrolase (EC-3.2.1.91) CBHI or CBHII cellulose-binding domain peptide from Trichoderma reesei, and cellulose is used as the adsorbent for fusion protein purification. The fusion protein may be treated with a specific protease (Factor-Xa, collagenase (EC-3.4.24.3), enterokinase (EC-3.4.21.9) or thrombin (EC-3.4.21.5) to separate the affinity tail. Abnormal folding of the recombinant protein does not occur in the resultant fusion protein, and cleavage hindrance due to steric hindrance does not occur. Inexpensive cellulose is used as the adsorbent. In an example, a beta-galactosidase (EC-3.2.1.23) fusion proteins were produced using plasmid pKE301-lacZ and plasmid pKE311-lacZ, with Escherichia coli JM109 as a host. (14pp)

L4 ANSWER 10 OF 12 EMBASE COPYRIGHT (c) 2007 Elsevier B.V. All rights reserved on STN
ACCESSION NUMBER: 2003021756 EMBASE
TITLE: Fusion of family VI cellulose binding domains to Bacillus halodurans xylanase increases its catalytic activity and substrate-binding capacity to insoluble xylan.
AUTHOR: Mangala S.L.; Kittur F.S.; Nishimoto M.; Sakka K.; Ohmiya K.; Kitaoka M.; Hayashi K.
CORPORATE SOURCE: K. Hayashi, Applied Enzymology Laboratory, National Food Research Institute, Tsukuba 2-1-2, Kannondai, Ibaraki 305-8642, Japan. khayashi@nfri.affrc.go.jp
SOURCE: Journal of Molecular Catalysis B: Enzymatic, (17 Feb 2003) Vol. 21, No. 4-6, pp. 221-230.
Refs: 30
ISSN: 1381-1177 CODEN: JMCEF8
PUBLISHER IDENT.: S 1381-1177(02)00226-6
COUNTRY: Netherlands

DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology: Bacteriology, Mycology, Parasitology and Virology
LANGUAGE: English
SUMMARY LANGUAGE: English
ENTRY DATE: Entered STN: 29 Jan 2003
Last Updated on STN: 29 Jan 2003

AB A tandem repeat of the family VI cellulose binding domain (CBD) from Clostridium stercorarium xylanase (XylA) was fused at the carboxyl-terminus of Bacillus halodurans xylanase (XylA). B. halodurans XylA is an enzyme which is active in the alkaline region of pH and lacks a CBD. The constructed chimera was expressed in Escherichia coli, purified to homogeneity, and then subjected to detailed characterization. The chimeric enzyme displayed pH activity and stability profiles similar to those of the parental enzyme. The optimal temperature of the chimera was observed at 60.degree.C and the enzyme was stable up to 50.degree.C. Binding studies with insoluble polysaccharides indicated that the chimera had acquired an increased affinity for oat spelt xylan and acid-swollen cellulose. The bound chimeric enzyme was desorbed from insoluble substrates with sugars and soluble polysaccharides, indicating that the CBDs also possess an affinity for soluble sugars. Overall, the chimera displayed a higher level of hydrolytic activity toward insoluble oat spelt xylan than its parental enzyme and a similar level of activity toward soluble xylan.

L4 ANSWER 11 OF 12 EMBASE
ACCESSION NUMBER: 1999131465 EMBASE
TITLE: Nucleotide sequences of two contiguous and highly homologous xylanase genes xynA and xynB and characterization of XynA from Clostridium thermocellum.
AUTHOR: Hayashi H.; Takehara M.; Hattori T.; Kimura T.; Karita S.; Sakka K.; Ohmiya K.
CORPORATE SOURCE: K. Sakka, Faculty of Bioresources, Mie University, Tsu 514-8507, Japan. sakka@bio.mie-u.ac.jp
SOURCE: Applied Microbiology and Biotechnology, (1999) Vol. 51, No. 3, pp. 348-357.
Refs: 41
ISSN: 0175-7598 CODEN: AMBIDG
COUNTRY: Germany
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology: Bacteriology, Mycology, Parasitology and Virology
LANGUAGE: English
SUMMARY LANGUAGE: English
ENTRY DATE: Entered STN: 10 May 1999
Last Updated on STN: 10 May 1999

AB A 5.7-kbp region of the Clostridium thermocellum F1 DNA was sequenced and found to contain two contiguous and highly homologous xylanase genes, xynA and xynB. The xynA gene encoding the xylanase XynA consists of 2049 bp and encodes a protein of 683 amino acids with a molecular mass of 74 511 Da, and the xynB gene encoding the xylanase XynB consists of 1371 bp and encodes a protein of 457 amino acids with a molecular mass of 49 883 Da. XynA is a modular enzyme composed of a typical N-terminal signal peptide and four domains in the following order: a family-11 xylanase domain, a family-VI cellulose-binding domain, a dockerin domain, and a NodB domain. XynB exhibited extremely high overall sequence homology with XynA (identity 96.9%), while lacking the NodB domain present in the latter. These facts suggested that the xynA and xynB genes originated from a common ancestral gene through gene duplication. XynA was purified from a recombinant Escherichia coli strain and characterized. The purified enzyme was highly active toward xylan; the specific activity on oat-spelt xylan was 689 units/mg protein. Immunological and zymogram

analyses suggested that XynA and XynB are components of the C. thermocellum F1 cellulosome.

L4 ANSWER 12 OF 12 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on STN

ACCESSION NUMBER: 2007:1072678 SCISEARCH
THE GENUINE ARTICLE: 214ZB
TITLE: Strategy for selecting and characterizing linker peptides for CBM9-tagged fusion proteins expressed in Escherichia coli
AUTHOR: Kavoosi, Mojgan; Creagh, A. Louise; Kilburn, Douglas G.; Haynes, Charles A. (Reprint)
CORPORATE SOURCE: Univ British Columbia, Michael Smith Labs, Vancouver, BC V6T 1Z3, Canada (Reprint); Univ British Columbia, Dept Chem & Biol Engn, Vancouver, BC V6T 1Z3, Canada; Univ British Columbia, Dept Microbiol & Immunol, Vancouver, BC V5Z 1M9, Canada
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ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The influence of linker design on fusion protein production and performance was evaluated when a family 9 carbohydrate-binding module (CBM9). serves as the affinity tag for recombinant proteins expressed in Escherichia coli. Two bioinformatic strategies for linker design were applied: the first identifies naturally occurring linkers within the proteome of the host organism, the second involves screening peptidases and their known specificities using, the bioinformatics software MEROPS (TM) to design an artificial linker resistant to proteolysis within the host. Linkers designed using these strategies were compared against traditional poly-glycine linkers. Although widely, used, glycine-rich linkers were found by tandem MS data to be susceptible to hydrolysis by E. coli peptidases. The natural (PT)(x)P and MEROPS (TM)-designed S3N10 linkers were significantly more stable, indicating both strategies provide a useful approach to linker design: Factor X, processing of the fusion proteins depended. strongly on linker chemistry, with poly(G) and S3N10 linkers showing the fastest cleavage rates. Luminescence resonance energy transfer studies, used to measure average distance of separation between GFP and Tb(III) bound to a strong calcium-binding site of CBM9, revealed that, for a given linker chemistry, the separation distance increases with increasing linker length. This increase was particularly large for poly(G) linkers, suggesting that this linker chemistry adopts a hydrated, extended configuration that makes it particularly susceptible to proteolysis. Differential scanning calorimetry studies on the PT linker series showed that fusion of CBM9 to GFP did not alter the To, of GFP but did result in a destabilization, as seen by both a decrease in T-m, and Delta H-cal, of CBM9. The, degree of destabilization increased :with decreasing length of the (PT)(x)P linker such that Delta T-m=-8.4 degrees C for the single P linker.

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L2            71 DUP REM L1 (12 DUPLICATES REMOVED)
L3             2 S L2 AND SOLUBLE PROTEIN
L4            12 S L2 AND (XYLANASE OR ENTEROKINASE)

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